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(71) Applicant *(for all designated States except US)*: ELI LILLY AND COMPANY [US/US]; Lilly Corporate Center, Indianapolis, IN 46285 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: KUHFELD, Michael, T. [US/US]; 5430 North Delaware Street, Indianapolis, IN 46204 (US). ZYNGER, Jacob [US/US]; 5017 Adams Boulevard, Indianapolis, IN 46220 (US). HINSHAW, Michael, E. [US/US]; 527 Nuthatch Drive, Zionsville, IN 46077 (US). STRATFORD, Robert, E. [US/US]; 13876 Perrin Drive, Carmel, IN 46032 (US). OSBORNE, Stacy, J. [US/US]; 3719 Wild Ivy Drive, Indianapolis, IN 47906 (US). BARLOW, Daryl, T. [US/US]; 5204 East County Road, 100 North, Danville, IN 47334 (US). BELL, Jeff, A. [US/US]; 3800 North Mohr Road, Greenfield, IN 46140 (US).

(74) Agent: SARUSSI, Steven, J.; McDonnell Boehnen Hulbert & Berghoff, Ltd., 7th floor, 300 South Wacker Drive, Chicago, IL 60606 (US).

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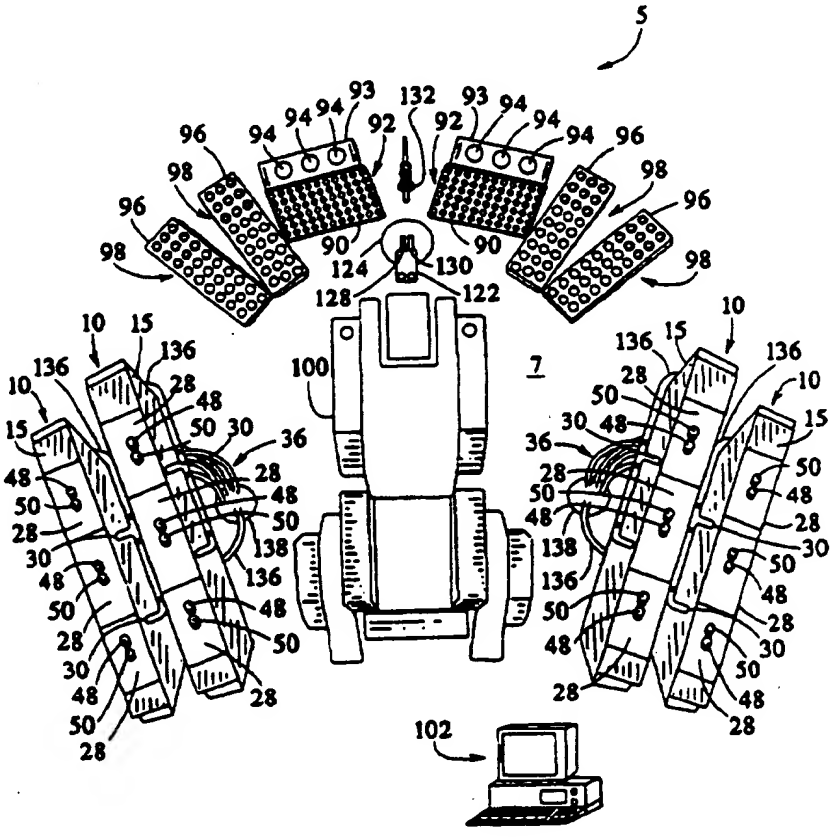
(57) Abstract

The present invention relates to an automated permeability analysis system that increases the capacity, precision, accuracy and reliability of *in vitro* drug candidate permeability studies. The inventive permeability analysis system establishes a working environment around which a robotic arm may maneuver to enable a computer to establish and carry out a multitude of simultaneous drug transport experiments, with minimal test operator involvement. The system thereby may be used on a relatively large scale to easily and accurately investigate mechanisms of drug transport across a variety of cell membranes and tissues that act as barriers to drug absorption. In particular, the system may be used with monolayers of cultured epithelial cells.



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AUTOMATED PERMEABILITY ANALYSIS SYSTEM

BACKGROUND OF THE INVENTION

The present invention relates to a system for conducting drug candidate permeability studies, and, more particularly, a system for investigating the transport characteristics of drug candidates using *in vitro* models.

Cultured cell systems have been used in the past to study drug transport across specific biological barriers. Human colon cancer cells (CaCO-2), for example, have been cultured on filters and used with test chambers to create an *in vitro* model to study intestinal absorption of particular drug candidates. Drug candidates such as aspirin, acetaminophen, cimetidine, ranitidine , and nizatidine, for example, have all been studied in such models. Other types of absorption kinetic studies, such as buccal, sublingual, and other epithelial surfaces for example, are conducted in a similar manner.

Studies of this general nature are described, for example, by: U.S. Pat. No. 5,183,760; Kuhfeld, M.T., chen, J., Hu, M. and Stratford, R.E., *Design of a Novel Diffusion Apparatus. Compatible with Millicell®Permeable Supports and its Characterization using CACO-2 Cells,* PHARM RES., v. 10, no. 10 (Oct. 1993) S-179; Adson, A., Raub, T.J., Burton, P.S., Barshun, C.L., Hilgers, A.R., Audus. K.L. and Ho, N.F.H., *Quantitative Approaches to Delineate Peracellular Diffusion in Cultured Cell Monolayers,* J. PHARM. SCI., 83 (1994) 1529-35; Anderson, B.W., Levine, A.S., Levitt, D.G., Kneip, J.M. and Levitt, M.D., *Physiological Measurement of Luminal Stirring in Perfused Rat Jejunum,* AM. J. PHYSIOL,, 254 (1988) G843-48; Artursson, P., Ungell, A. and Lofroth, J., *Selective ParacellularPermeability in Two Models of Intestinal Absorption: Cultured Monolayers of Human Intestinal Epithelial Cells and Rat Intestinal Segments,* PHARM. RES., 10 (1993); Banakar, U.V., *Introduction. Historical Highlights, and the Need for Dissolution Testing,* In Banakar, U.V. (Ed), Pharmaceutical Dissolution Testing, Marcel Dekker, New York (1992) pp. 1-18; Banakar, U.V., *Theories of dissolution,* In Banakar, U.V. (Ed), Pharmaceutical Dissolution Testing, Marcel Dekker, New York (1992) pp. 19-51; Barry, P.H. and Diamond, J.M., *effects of Unstirred Layers on Membrane Phenomena,* PHYSIOL, REV., 64 (1984) 763-872; Burr, A. and Mork, N., *Metabolism of Testosterone During In Vitro Transport Across Caco-2 CellMonolayers: Evidence for Beta-Hydroxysteroid Dehydrogenase Activity in Differentiated Caco-2Cells,* PHARM RES., 9 (1992) 1290-94; Chandler, E.C., Zaccaro, L.M.

and Moberly, J.B., *Transepithelial Transport of Cholyltaurine by Caco-2 Cell Monolayers is Sodium Dependent*, AM. J. PHYSIOL, 264 (1993) G1118-25; Coburn, J.N., Donovan, M.G. and Schasteen, C.S., *A Model of Human Small Intestinal Absorptive Cells. 1. Transport Barrier*, PHARM RES., 8(1991) 210-16; Dantzig, A.H., Duckworth, D.C. and Tabas L.B., *Transport Mechanism Responsible for the Absorption of Loracarbef, Cefixime, and Cefuroxime Axetil into Human Intestinal Caco-2 Cells*, BIOCHEM BIOPHYS ACTA, 1191 (1994) 7-13; Dietschy, J.M. and Westergard, H., *The Effect of Unstirred Water Layers on Various Transport Processes in the Intestine*, In Csaky, T.Z. (Ed), *Intestinal Absorption and Malabsorption*, Raven, New York (1975) pp. 197-206; Hidalgo, I.J., Raub, T.J. and Borchardt, R.T., *Characterization of the Human Colon Carcinoma Cell Line (Caco-2) as a Model System for Intestinal Epithelial Permeability*, GASTROENTEROLOGY 96 (1989) 736-749; Hidalgo, I.J., Hillgren, K.M., Grass, G.M. and Borchardt, R.T., *Characterization of the Unstirred Water Layer in Caco-2 Cell Monolayers Using a Novel Diffusion Apparatus*, PHARM RES., 8 (1991) 222-27; Hu, M., Sinko, P.J., DeMeere, A.L.J., *Johnson, D.A. and Amidon, G.L. Membrane Permeability Parameters for Some Amino Acids and Beta-Lactam Antibiotics of the Boundary Layer Approach*, J. THEOR. BIOL., 131 (1988) 107-14; Hu, M. and Borchardt, R.T., *Mechanism of L-Alpha-Methyldopa Transport Through a Monolayer of Polarized Human Intestinal epithelial Cells (Caco-2)*, PHARM RES., 7 (1990) 1313-19; Hu, M. and Borchardt, R.T., *Transport of a Large Neutral Amino Acid in a Human Intestinal Epithelial Cell Line (Caco-2)*: Uptake and Efflux of Phenylalanine, BIOCHEM BIOPHYS ACTA. 1135 (1992) 233-44; Hu, M., Chen, J., Tran, D. Zhu, Y. and Leonardo, G., *The Caco-2 Cell Monolayers as an Intestinal Metabolism Model; Metabolism of Dipeptide Phe-pro*, J. DRUG TARGETING, 2 (1994a) 79-89; Hu, M., chen, J., Zhu, Y., Dantzig, A.H., Stratford, R.E. and Kuhfeld, M.T., *Mechanism and Kinetics of Transcellular Transport of a New Beta-Lactam Antibiotic Loracarbef Across an Intestinal Epithelial Membrane Model System (Caco-2)*, PHARM RES., 11 (1994b) 1405-1413; Karlsson, J. and Artursson, P., *A Method for the Determination of Cellular Permeability Coefficients and Aqueous Boundary Layer Thickness in Monolayers of Intestinal Epithelial (Caco-2) Cells Grown on Permeable Filter Chargers*, INT. J. PHARM, 71(1991) 55-64; Karlsson, J. and Artursson, P., *A New Diffusion Chamber System for the Determination of Drug, Permeability Coefficients Across the Human Intestinal Epithelium that tare Independent of the Unstirred Water Layer*, BIOCHEM BIOPHYS ACTA, 1111 (1992) 204-10; Komiya, I., Park, J.Y., Kamani, A., Ho, N.F.H. and

Higuchi, W.I., *Quantitative Mechanistic Studies in Simultaneous Fluid Flow and Intestinal Absorption Using Steroids as Model Solutes*, INT. J. PHARM, 4 (1980) 249-62; Kramer, C.Y., *Extension of Multiple Range Tests to Group Means with Unequal Numbers of Replications*, BIOMETRICS 12 (1956) 309-10; Leuenberger, H., Buchman, S., Reinke, C. and Schmid, B., *An In Vitro Absorption Model System Based on Cell Monolayers*, In Wilson. G., Illum, L., Davis, S.S. and Zweibaum, A. (Eds), Pharmaceutical Applications of Cell and Tissue Culture to Drug Transport, NATO ASI Series, Plenum, New York (1991) pp. 121-139; Ma, T.Y., Dyer, D.L. and Said, H.M., *Human Intestinal Cell Line Caco-2; A useful Model for Studying Cellular and Molecular Regulation of Biotin Uptake*, BIOCHIM BIOPHYS ACTA, 1189 (1994) 81-88; Shah P.K., Hidalgo, I.J. and Borchardt, R.T., *A simple Diffusion Device to Study Transport Across Cells Cultured on Microporous Membranes*, INT. J. PHARM, 63 (1990) 281-83; Strocchi, A. and Levitt, M.D., *A Reappraisal of the Magnitude and Implication of the Intestinal Unstirred Layer*, GASTROENTEROLOGY 101 (1991) 843-847; Thwaites, D.T., Brown, C.D.A., Hirst, B.H. and Simmons, N.L., *H + Coupled Dipeptide (glyclysarcosine) Transport Across Apical and Basal Borders of Human Intestinal Caco-2 Cell Monolayers Display Distinctive Characteristics*, BIOCHIM BIOPHYS ACTA, 1151 (1993) 237-45; Winnie, D., *Unstirred Layer as a Diffusion Barrier In Vitro and In Vivo*, In Skadhauge, E. and Heinize, K. (Eds), Intestinal Absorption and Secretion, MTP Press, Lancaster (1984) pp. 21-38; and Zheng, L., chen, J., Zhu, Y., Yang, H., Elmquist, W. and Hu M., *Comparison of the Transport Characteristics of D- and L-methionine in a Human Intestinal Epithelial Model (Caco-2) and in a Perfused Rat Intestinal Model*, PHARM RES., 11 (1994) 1771-76; the disclosures of which are incorporated herein by reference.

All such drug candidate transport studies typically are conducted in a manual fashion, whereby the substantial involvement of a test operator is required to ensure that the experiment is established and carried out in a timely and accurate manner. The experiments often prove to be quite labor intensive and time consuming in this regard. Moreover, such demands on the test operator escalate significantly when two or more drug transport experiments are being conducted simultaneously using multiple test chambers. The ever increasing need to rapidly and accurately assess the *in vitro* permeability of a series of compounds (or analogs within a series), however, demands a reliable, accurate, and high volume screening procedure.

It is therefore an object to the present invention to provide a system that significantly reduces the demands imposed on a test operator.

Another object of the present invention is to provide a system that increased the capacity, accuracy, and precision of drug candidate permeability studies.

It is also an object of the present invention to provide a system that can more quickly generate a larger amount of drug candidate permeability data to expedite drug candidate screening procedures.

Other objects of the present invention will be apparent by the following description.

SUMMARY OF THE INVENTION

The automated permeability analysis system of the present invention meets these and other objectives, thereby providing a variety of benefits over earlier transport study methods and systems.

The inventive permeability analysis system establishes a working environment around which a robotic arm may maneuver to enable a computer to establish and carry out a multitude of simultaneous drug transport experiments, with minimal test operator involvement. The system thereby may be used on a relatively large scale to easily and accurately investigate mechanisms of drug transport across a variety of cell membranes and tissues that act as barriers to drug absorption. The system can also support an SAR for co-optimization of drug activity and drug bioavailability. Moreover, the system similarly may be used as a screen for oral bioavailability in support of combinatorial chemistry approaches, insofar as there exists the potential for relative measurements of transport rates from stoichiometric mixtures of related compounds.

BRIEF DESCRIPTION OF THE DRAWING

A preferred embodiment of the present invention is described herein with reference to the drawing, wherein:

FIGURE 1 is a plan view of various table-top components of the preferred automated permeability analysis system, including a robotic arm at the center of the table;

FIGURE 2 is a front view of the robotic hand that is attached as a part of the robotic arm shown in Figure 1;

FIGURE 3 is a bottom view of the robotic hand that is shown in Figure 2 and attached as a part of the robotic arm shown in Figure 1;

FIGURE 4 is a schematic representation of additional components of the present invention located below the work table top;

FIGURE 5 is a cross-sectional schematic view of the preferred diffusion chamber that is used as a part of the present invention;

FIGURE 6 is an elevational view through the diffusion chamber shown in Figure 5 from the donor side, with the donor side end cap removed;

FFIGURE 7 is a side view of the preferred cell culture insert loading tool that is used as a part of the present invention to insert and remove filters from the diffusion chamber shown in Figures 5 and 6;

FIGURE 8 is a perspective view of the insert loading tool shown in Figure 7;

FIGURE 9 is an elevational view of one chamber bank, having three diffusion chambers positioned by cradles within a water trough above three magnetic stirring devices;

FIGURE 10 is a perspective view of one of six cradles that are used in a chamber bank to properly position and maintain the diffusion chambers within the water trough; and

FIGURE 11(a) and 11(b) together present a flow chart diagram illustrating the operation of the automated test sequence of the present invention.

DESCRIPTION OF THE PREFERRED EMBODIMENT

Referring now in detail to the various figures of the drawing, there is shown a preferred automated permeability analysis system of the present invention.

Figure 1, for example, is a plan view of various components of the preferred automated permeability analysis system **5**

In certain embodiments , the permeability analysis system 5 is disposed within an enclosure capable of preventing light from contacting the samples that are to be analyzed. The enclosure must, of course, be large enough to allow the complete range of motion of robotic arm 100. The enclosure will preferably be used in situations where compounds to be analyzed are light sensitive.

Preferably , a 4' x 6' table top **7** acts as the work surface for the apparatus and experiments. Positioned on the table top surface are, for example, four chamber banks **10**. As is best shown in Figure 9, each chamber bank **10** has a circulating water trough **15** formed from a polycarbonate material such as, for example, Lexan®. The troughs **15** are fed through water feed lines **136**. In particular, each of the two lines **136** passes through an aperture **138** from beneath the surface of the table **7**, and divides to serve a subset of the chamber banks, e.g., two chamber banks **10**. Each chamber bank **10** includes two ports **30** through which water is received from a common branch of the divided feed line **136**. The trough **15** is discharged through water falls **34** at either end of the chamber bank **10.** The smaller the size of the water trough **15**, the easier it generally is to control and maintain the temperature of the water.

Each chamber bank **10** also has a plurality of, e.g., three, diffusion chambers **20** situated within the water trough **15** above three magnetic stirring devices **25**. The magnetic stirring devices **25** are configured such that the water trough **15** may rest on top of the series of three stirring devices **25**. Situated within each stirring device **25** adjacent the bottom of the water trough **15** is a stirring motor having sufficient power to ensure proper mixing within the diffusion chamber **20**.

Attached, e.g., glued to the inside surface of the water trough **15** are cradles **16** into which the diffusion chambers **20** are removably secured to ensure proper positioning of each chamber **20** in the chamber bank **10**. Once the diffusion chambers **20** are properly positioned in the respective cradles **16**, each station in the chamber bank **10** can be capped with a lid **28**,

which is also formed a polycarbonate material such as from Lexan®, to reduce evaporation from the water trough 15.

The individual diffusion experiments are conducted within the diffusion chambers 20. The preferred diffusion chamber 20, which is best shown for example in Figures 5 and 6, is a unibody design constructed from Lexan®. The Lexan® material does not tend to bind to test compounds, is extremely rugged, and is rather easy to machine. Moreover, transparent Lexan® components, including the diffusion chamber 20, may be obtained by dipping the components in fumes of boiling chloroform. A transparent diffusion chamber permits observation of the experiment as the test progresses.

Figure 5 is a schematic cross-sectional view of the preferred diffusion chamber 20. The diffusion chamber 20 comprises a unibody portion 39 in which there is an apical or donor side 40 having a volume of approximately 14 mL, and a basolateral or receiver side 42 having a volume of approximately 13mL. During the experiment, the donor side 40 and the receiver side 42 are separated by a Millicell® PC or PCF culture plate insert device 44 having a 30 mm, 0.4 μ pore size filter 46. Sampling ports 48 and 50 fluidly communicate with the donor side 40 and the receiver side 42, respectively, and are of a sufficient height to extend through holes in the lids 28 so as to permit the robotic hand 104 to deliver and retrieve solutions from the diffusion chamber 20 without interfering with the lids 28. The sampling ports 48 and 50 are also beveled at their respective top edges 49 and 51 to cooperate with the pipette tips 92.

The ends of the diffusion chamber 20 are terminated with removable and interchangeable end caps 52 and 54, which each have a notch 17 to cooperate with a recess 18 of cradles 16 to secure the diffusion chamber 20 into position in the chamber bank 10. As is best shown in Figure 10, cradles 16 are glued on their sides and bottom to the water trough 15. For each diffusion chamber 20, two cradles 16 are secured in a spaced relationship within the water trough 15 to support the respective ends of the chamber 20. Each cradle 16 has two cut-out portions 19 to allow water to flow more easily past the cradle 16 within the water trough 16.

O-rings 56, 58 and 60 are used to seal the donor side 40 and the receiver side 42 of the diffusion chamber 20. Magnetic stir bars 62 and 64 rotate at a rate sufficient to adequately stir the chamber contents, e.g., 600 rpm, at the base of the diffusion chamber 20 in cooperation with the magnetic stirring devices 25 shown in Figure 9. The bottom exterior surface of the diffusion chamber 20 can be flattened, as is best shown for example in Figure 6, to help

stabilize the chamber **20** when resting on a flat surface, and to allow the stir bars **62** and **64** to more closely cooperate with the magnetic stirring devices **25** for greater stirring effect.

Figure 5 and 8 illustrate a loading tool **70** formed from Lexan® that is used to insert and remove culture plate insert devices **44**, including the associated filters **46**, from the diffusion chamber **20**. In particular, the larger end **72** of the tool **70** fits within the receive side **42** of the chamber **20** and is used before the experiment to push the culture plate insert device **44** into position through the receiver side **42** of the chamber **20**. To this end, the edge **74** of the tool 70 is configured to contact the bottom exterior surface of the culture plate insert device **44** at location **76** without disturbing the filter portion **46** of the device **44**. Channels **78** and 79 extend through the tool 70 as shown in Figures 7 and 8, and allow air pressure to be released through the tool **70** as the culture plate insert device **44** is inserted into position. This helps prevent any damage or other disruption to the filter **46** and any cells thereon.

Once the experiment is complete, the small end **80** of the tool **70** is inserted through the donor side **40** of the chamber **20** and is used to push the culture plate insert device **44** out of the diffusion chamber **20** through the receiver side **42**. The beveled edge **82** of the tool **70** is similarly configured to contact the bottom interior surface of the culture plate insert device **44** at location **84**, again without disturbing the filter portion **46** of the device **44**.

Referring once again to Figure 1, also situated on the table top are two pipet tip holder racks **90** to house up to a total of 144 pipette tips **92**, two source racks **93** to house six source vials **94** for sample or test solutions, and four sample vial racks **93** to house up to a total of 132 sample vials **98** in which donor and receiver samplings are collected for up to 12 simultaneous transfer experiments. Sample vials **98** preferably are constructed from glass.

Each of the 12 columns of sample vials **98** shown in Figure 1 corresponds to one of the twelve diffusion experiments being conducted. Moreover, the dark-colored vials represent sample vials **98** in which samplings from the donor side **40** of the diffusion chambers **20** are preferably kept during the experiment, while the light-colored vials represent sample vials **98** in which samplings from the receiver side **42** are preferably kept. Each of the receiver sample vials includes a flat-bottomed glass insert so that the receiver sample sorted within the vial is easier to retrieve from the vial for analysis. The donor sample vials do not require an insert in the preferred embodiment because the more concentrated donor samples are diluted within the vial before analysis, making retrieval easier.

Bolted onto the center of the table is a Mitsubitshi Movemaster EX robotic arm **100**, which has a range of movement around the surface of the table top of approximately 300 degrees. Computer **102** is programmed in a conventional manner, such as in GW BASIC, to control the various operations of the robotic arm **100**. The robotic arm **100** is used during the experiment to transfer liquids as required by the experiment. Therefore, the source vials **94**, the sample vials **98**, the pipette tips **92**, and the sampling ports **48** and **50** of al involved diffusion chambers **20** must be accessible to the robotic arm **100** during the experiment. To this end, the two chamber banks **10** immediately adjacent the robotic arm **100** are more elevated off the table top surface **7** than are the chamber banks **10** at the outer edges of the table.

A hand **104** is attached to the robotic arm **100** through a stainless steel circular disc block attachment piece **106**, as is shown for example in Figures 2 and 3. The disc **106** is attached to the robotic arm **100** for example with screws that pass through holes **107** in the disc **106**. The hand **104** itself includes a rectangular stainless steel block **108** in which there are bored three right-angled channels **110, 112** and **114** for the passage of air and/or liquids. Each of the three channels **110, 112** and **114** has a threaded opening at both the rear side and the bottom side of the stainless steel block **108**. Attached to the bottom side of the block **108** are two stainless cannulae **116** and **118**, which are in fluid communication with channels **110** and **114**, respectively. The two cannulae **116** and **118** are used to deliver buffers as required for the experiment.

Also attached to the bottom of the block **108** is one stainless steel pipet tip receiver **120**, which communicates with channel **112**. The pipet tip receiver **120** can pick up a disposable plastic pipet tip **92** by entering the upper opening of the pipet tip **92** to the point where a frictional fit is created between the inside surface of the pipet tip **92** and the outside surface of the pipet tip receiver **120**, as shown for example in Figure 2. The computer **102** controls the movements of the robotic arm **100** to ensure that the cannulae **116** and **118** of the robotic hand 104 do not obstruct or otherwise inhibit the pick-up or maneuvering of the pipette tip **92**.

A pipet tip extractor **122** is secured to the table top **7** and disposed over a hole **124** that leads to a waste bucket **126** below the table surface. A pipette tip **92** is removed from the robotic hand **104** by placing the pipette tip **92** between the two lines **128** and **130** of the extractor **122**, and then lifting up on the robotic arm **100**. The frictional fit between the

pipette tip 92 and the pipette tip receiver 120 is thereby relieved, and the pipette tip 92 falls into the waste bucket 126 below the table surface. A sensor 132 is positioned adjacent the hole 124, and is used by computer 102 to verify that the robotic arm 100 has either successfully picked-up or successfully disposed of a pipette tip 92.

Since the robotic arm 100 must move throughout the experiment to a number of defined locations, most table top components described above are removably secured to the table top 7 by pins so as to ensure proper positioning of the various components on the work surface 7 relative to the robotic arm 100.

Referring now to figure 4, located on a shelf below the surface of the work table are two VanKel heater/circulator water pumps, 134, which maintain a 37°C water temperature and circulate the water to the water troughs 15 through circulating lines 136 that pass through apertures 138 in the table top. The 37°C water temperature simulates the 37°C temperature of the human body. Two water collector devices 140 receive the water returning from the troughs 15 through return lines 36. The two respective VanKel pumps 134 are fed from the bottom of the water collector devices 140 so as to avoid the introduction of air pockets into the water system.

Also situated under the table top are the controller 142 for the robotic arm 100, two switch controls 144 that each turn on and off a series of, for example, six magnetic stirring devices, 25, the waste bucket 126 to receive pipette tip waste, and a printer 146 that communicates with the computer 102 to print reports. A WTI smart switch 148, model SS-8B, acts as a communications hub for the computer 102 and various other components of the system, such as, for example, the robotic arm 100, the printer 146, and other components described below.

Two Hamilton syringe/dilutors 150 and 152 below the table top communicate through lines 154, 155, and 156 with the three ports 160, 162, and 164 at the back side of the robotic hand 104. In particular, syringe/dilutor 150 contains two 5 mL syringes. The first of the two 5 mL syringes is used to deliver a pH 6 transport buffer to port 160 of the robotic hand 104 through fluid line 154. The pH 6 transport buffer is maintained at 37°C in water bath 141 the second of the two 5 mL syringes is used to deliver a pH 7.4 transport buffer to port 164 of the robotic hand 104 through fluid line 156. The pH 7.4 transport buffer is also maintained at 37°C in water bath 141. Syringe/dilutor 152 has one 250 μL syringe that communicates through air line 155 with port 162 of the robotic hand 104, and is used to provide either an air

flow or a vacuum at the pipette tip receiver **120** as necessary. The computer **102** works through the smart switch **148** to activate the various syringes of the syringe/dilutors **150** and **152** at the appropriate times.

A programmable logic controller board **166** below the table top includes two output chips and one input chip. The two output chips of the controller board **166** work with the smart switch **148** to provide communication between the computer board **166** work with the smart switch **148** to provide communication between the computer **102** and two computer-controlled flow valves **168** and **170**, which control fluid communication between the two syringes of the syringe/dilutor **150** and the respective ports on the robotic hand **104**. The pipette sensor **132** on the table top also communicates with the computer **102** through the input chip of the computer board **166**.

The preferred diffusion experiment strives to mimic the drug absorption process that occurs, for example, in the small intestine. Inside the small intestine, or lumen, is a tubular structure that is covered with polarized epithelial cells. Successful oral drug candidates pass from the lumen, across the epithelial cell barrier, into the blood stream.

Accordingly, for the preferred experiment human colon cancer cells (CaCO-2) are cultured on the filter portions **46** of the culture plate insert devices **44**. The cancer cells may be obtained, for example, from the ATCC (American Type Culture Collection, Rockville, MD). It will be apparent to those of skill in the art that alternative cell lines may instead be used to perform other types of studies. Endothelial cells, for example, may be used to study the transport of drug candidates across the blood/brain barrier.

The microporous culture plate insert devices **44** may be obtained, for example, from the Millipore Corporation. The culture plate insert devices **44**, with cells grown thereon, are first subjected to an electrical resistance barrier integrity check, and then manually loaded with the tool **70** into the various diffusion chambers **20**. The diffusion chambers **20** are then placed at the various stations in the water troughs **15** above the magnetic stirring devices **25**.

Referring now to the flow chart diagram presented by Figures 11(a) and 11(b), it is shown that the automated test sequence begins at block **174** where the computer system **102** and all associated communication links are initialized. Block **175-179** show that certain parameters may be inputted into the computer system **102**, including total duration of the experiment, the amount of sample/test solution to be withdrawn from which source vial, an identification of which stations are included in the overall experiment, and whether the fluid

lines 154 and 156 should either be flushed before the experiment commences, or washed at the conclusion of the experiment. Block 180 shows that fluid lines 154 and a56 are first flushed with the respective buffer solutions if previously requested in connection with block 179.

Once the system has been initialized and appropriately configured, the donor side 40 and receiver side 42 of each diffusion chamber 20 are filled with pH 6 and pH 7.4 transport buffers, respectively, as is represented by block 181 of the flow chart diagram. The pH of the lumen, where most of the absorption takes place, is 6, and the pH of blood is 7.4. The transport buffers are placed by the cannulae 116 and 118 of the robotic hand 104 into the diffusion chambers 20 through the two sampling ports 48 and 50. The cannulae 116 and 118 are therefore preferably positioned on the robotic hand 104 so as to permit simultaneous transfer of the transport buffers into the respective sides of the diffusion chamber 20. Simultaneous introduction of the transport buffers into the diffusion chamber 20 tends to equalize the pressures within the chamber 20, thereby helping to prevent any disruption to the filter 46.

Referring now to blocks 182-186, each station is first set up by securing a clean pipette tip 92 on the robotic hand 104, and drawing up to 400 μL of a test compound from a source vial 94 into the pipette tip 92 by a vacuum generated by the syringe/dilutor 152. The test compound within the pipette tip 92 is then delivered to the donor side 40 at the particular test station, and the delivery time is recorded. Three initial donor samples are taken with a clean pipette tip 92, the pipette tip 92 being first wetted before sampling. The three initial donor samples are delivered to three separate sample vials 98, each pipette tip 92 being touched off at the end of each delivery. The computer 102 then calculates, based upon the test compound delivery time, when samples should be taken from the receiver side 442 during the course of the experiment.

The preferred sample times for a two-hour experiment, for example, would be at 30, 60, 90, and 120 minutes as measured from the original test compound delivery time. For a one-hour experiment, on the other hand, the preferred sampling times would be at 15, 30, 45, and 60 minutes as measured from the original test compound delivery time.

The entire process is repeated until the donor side 40 at each test station contains the appropriate test compound for the experiment. During this time it may become necessary to

take a receiver sample form one of the stations, depending upon the time tables calculated in connection with block 186.

Blocks 182 and 187-192 of Figure 11(a) relate to the measurement, as a function of time, of how much of the test compound in each diffusion chamber 20 is transported (i.e., permeates) from the donor side 40 through the cell barrier into the buffer on the receiver side 42. In the preferred procedure, such measurements are made by using clean pipette tips 92 to periodically sample approximately 200 μL of the solution on the receiver side 42 during the course of a study that may last, for example, two hours. The time is recorded each time a sample is taken. The resulting samples are stored in individual clean sample vials 98, each pipette tip 92 being touched off at the end of each delivery. After a sample is taken, the volume of solution withdrawn from the receiver side 42 is replaced with pH 7.4 buffer so as to maintain a constant volume. Once it is determined that the last receiver sample has been taken for a particular station, two final donor samples are individually taken with clean, wetted pipette tips 92, and delivered to clean sample vials 98, as is shown for example by blocks 190 and 191. Again, the pipette tips 92 are each touched off after each delivery.

The computer 102 is configured to carry out all of these delivery and sampling procedures at the appropriate time intervals, as dictated by the time tables established in connection with block 186.

Referring now to block 192 of Figure 11(a), the sampling routines conclude once it is determined that all test compound experiments have been completed at all stations. The samples placed in the sample vials 98 during the course of the experiment may then be analyzed to determine the permeability of test compounds. Such analysis may include, for example, the use of high pressure liquid chromatography (HPLC) with fluorescence detection. Spreadsheet software programs may be used as a part of the data analysis, and may include an identification of the total length of the experiment, the sample amount, the source vial, and the particular sampling times and results for each station involved in the experiment.

The preferred test procedure then continues for the purpose of determining whether any of the stations experienced an unacceptable amount of leakage during the course of the experiment. Such leakage may result from, for example, a filter 46 that was damaged when the culture plate insert device 44 was installed in the diffusion chamber 20. The check is preferably made using the leakage marker check procedure that is shown for example in Figure 11(b) and described below.

The source vials 94 of test compound are first placed with source vials 94 containing a solution of Molecular Probes SR101 leakage marker. Referring not to Figure 11(b), the leakage marker check is set up by using clean pipette tips 92 to add up to 400 μL of leakage marker solution to the donor side 40 of each diffusion chamber 20 used in the test compound experiment. For each diffusion chamber 20, the leakage marker solution is preferably obtained from the same source vial 94 position as was the original test compound. The delivery time of the leakage marker solution is recorded, and the computer 102 calculates, based upon the delivery time, when samples should be taken from the diffusion chamber 20 during the course of the leakage marker test.

Blocks 196-198 of Figure 11(b) show that samples are taken at the appropriate time intervals from both the receiver side 42 and the donor side 40. In particular, the preferred sampling occurs between 20 and 30 minutes after delivery of the leakage marker solution to the donor side 40, whereby clean pipette tips 92 are used to take on 200 μL sample from the receiver side 42 and on 100 μL sample from the donor side 40 at each station. Individual samples are deposited in individual clean sample vials 98. Again, the pipette tips 92 are wetted and touched off during this procedure.

The purpose of the sampling is to determine how much of the leakage marker solution passes from the donor side 40 to the receiver side 42 at each station during the test period. If subsequent analysis to the receiver sampling evidences an unacceptably high level of transferred leakage marker solution for one or more diffusion chambers 20, then the test compound permeability results corresponding to these particular diffusion chambers 20 can be either carefully scrutinized or disregarded altogether.

Once again, the computer 102 is configured to carry out all of these delivery and sampling procedure at the appropriate time intervals, as dictated by the time tables established in connection with block 195.

At the conclusion of the leakage marker test, the two syringes of syringe/dilutor 150, as well as lines 154 and 156, are automatically washed with clean water contained in the rinse flask 172 if such an option was originally selected in connection with block 179. The culture plate insert devices 44 are also manually unloaded from the diffusion chambers 20. The diffusion chambers 20 clean-up rather quickly and easily as a result of their unique design.

It will be apparent to those of ordinary skill in the art that the automated permeability analysis system disclosed and describe herein can be modified without departing from the true spirit and scope of the invention.

WHAT IS CLAIMED:

1. A device for determining drug permeability characteristics across a biological barrier comprising

(a) a plurality of diffusion chambers, the diffusion chambers having an apical portion adjacent a basolateral portion and a culture plate removably disposed between the apical and basolateral portions; and

(b) an automated means for providing compounds to the apical portion and obtaining samples from the basolateral portion.

2. A device according to Claim 1, further comprising a means for maintaining isothermal conditions about the diffusion chambers.

3. A device according to Claim 1, wherein the culture plate includes a filter portion.

4. A device according to Claim 3, wherein cells are disposed on the filter portion.

5. A device according to Claim 1, wherein the automated means for providing compounds comprises a computer controlled means for transferring liquid samples.

6. A device according to Claim 4, wherein the computer controlled means for transferring samples comprises a robotic arm.

7. A device according to Claim 4, wherein the robotic arm includes a hand having at least one port for the passage of gas or fluid.

8. A device according to Claim 1, wherein a plurality of the the diffusion chambers are removably mounted in a chamber bank.

9. A device according to Claim 1, wherein the chamber banks are formed from polycarbonate.

10. A device according to Claim 1, wherein a magnetic stirring device is disposed beneath at least one diffusion chamber.

11. A device according to Claim 1, wherein the means for maintaining isothermal conditions comprises a water troughs provided in the chamber banks.

12. A device according to Claim 1, wherein the device is situated on a table surface.

13. A device according to Claim 6, wherein the robotic arm has a 300° range of motion about the table surface.

14. A device according to Claim 1, wherein the diffusion chambers are formed from polycarbonate.

FIG. 1

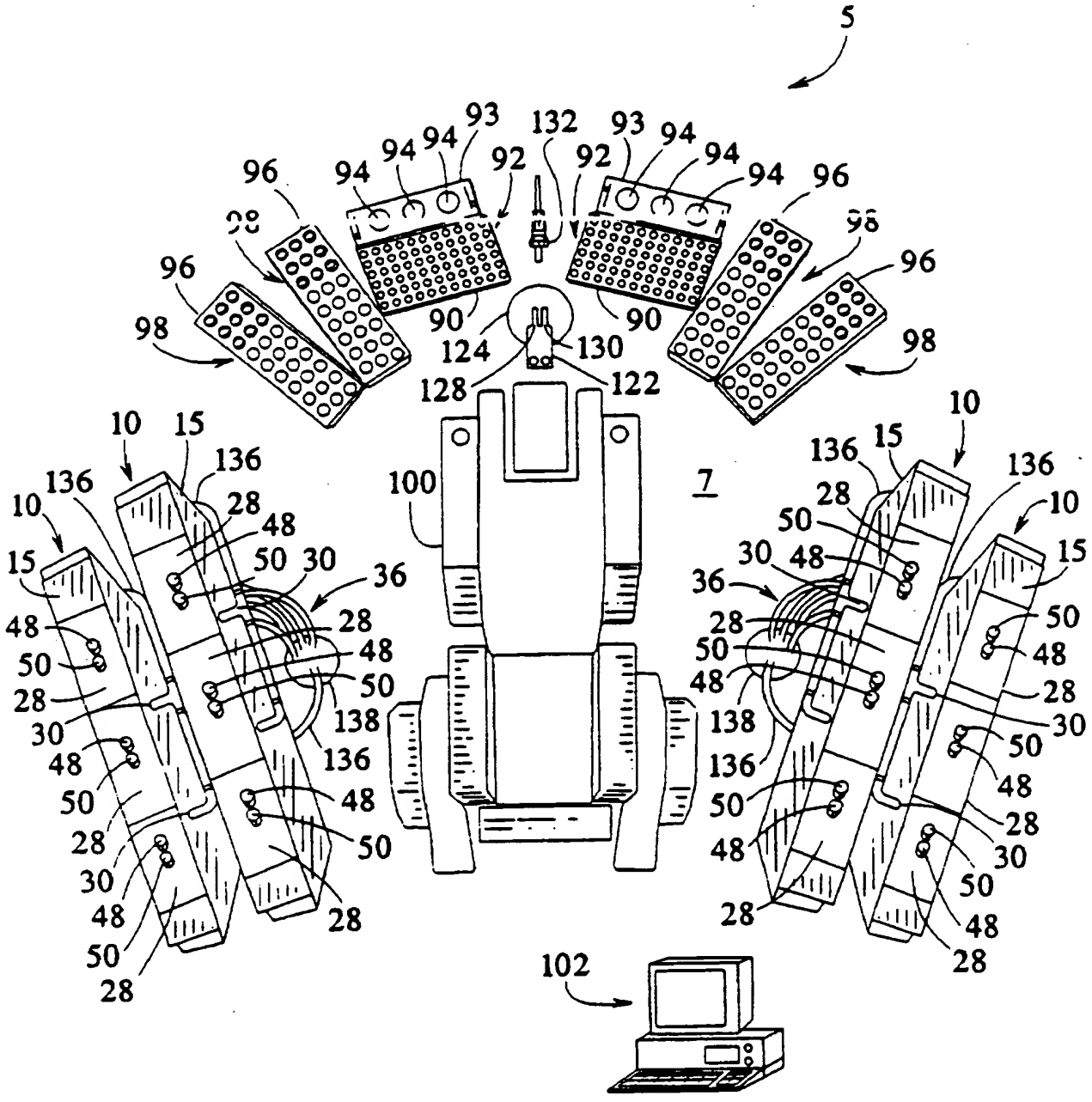
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FIG. 2

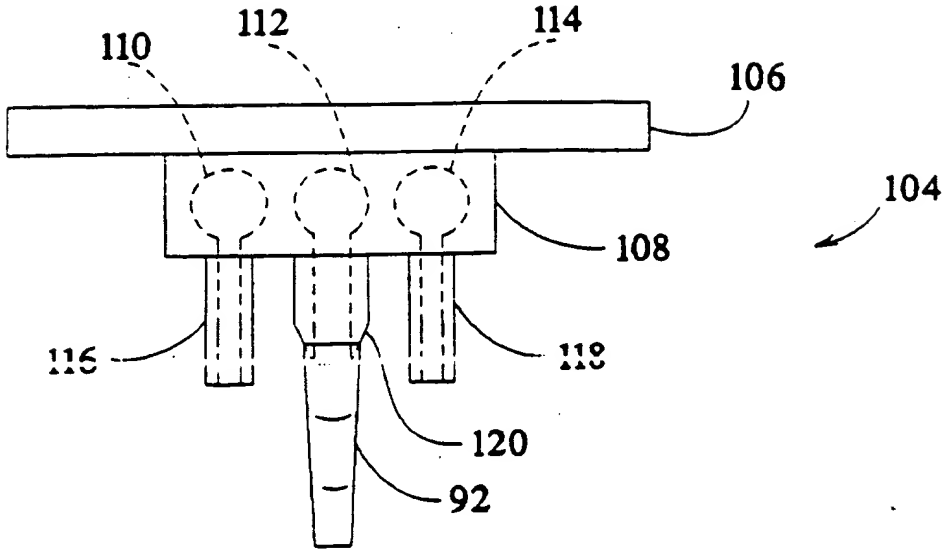
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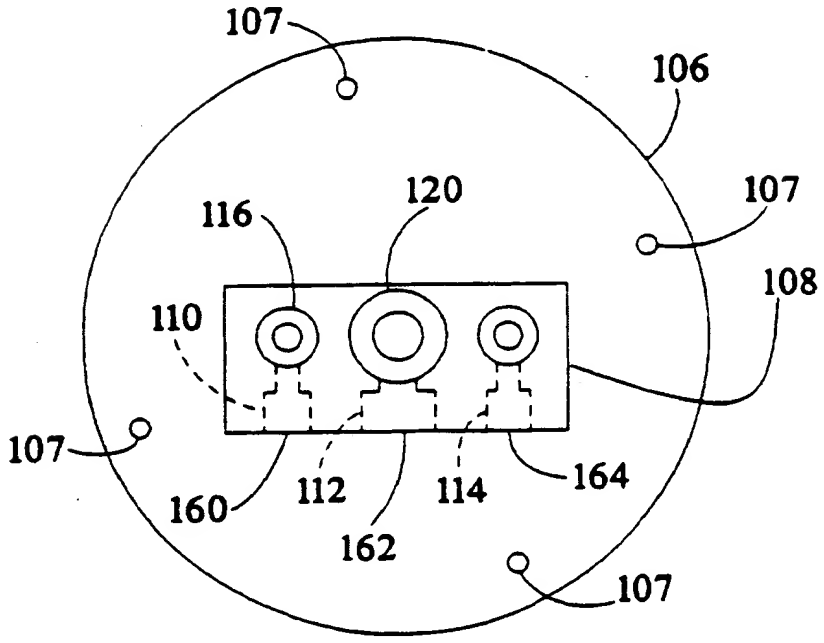
FIG. 3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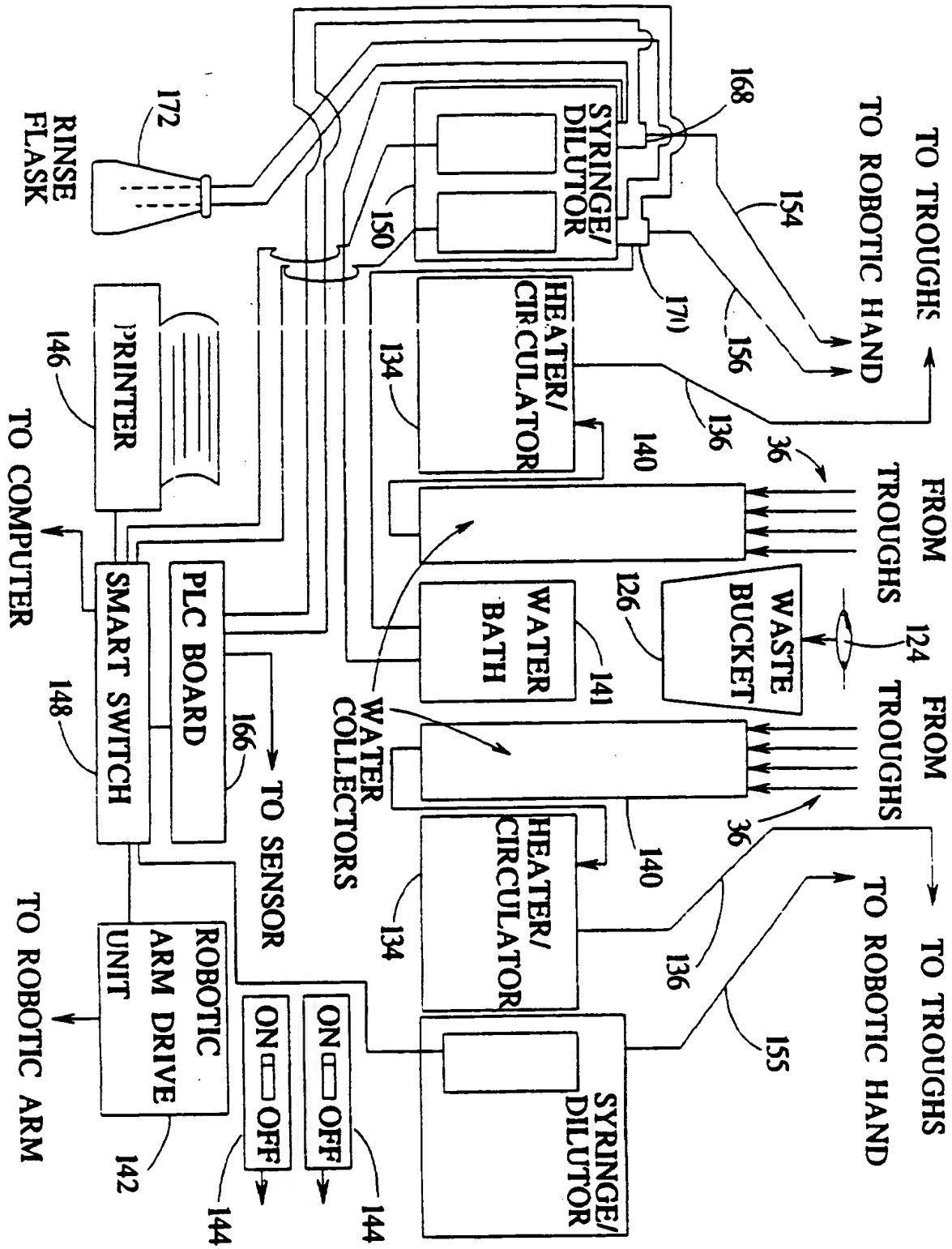
TO TROUGHS
TO ROBOTIC HAND
SYRINGE/ DILUTOR
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172
RINSE FLASK
154
150
171
156
HEATER/ CIRCULATOR
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140
36
FROM TROUGHS
WASTE BUCKET
126
124
FROM TROUGHS
TO TROUGHS
TO ROBOTIC HAND
PRINTER
146
TO COMPUTER
WATER BATH
141
WATER COLLECTORS
SMART SWITCH
148
PLC BOARD
166
TO SENSOR
HEATER/ CIRCULATOR
134
140
36
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155
SYRINGE/ DILUTOR
ROBOTIC ARM DRIVE UNIT
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TO ROBOTIC ARM
ON OFF
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FIG. 4

FIG. 5

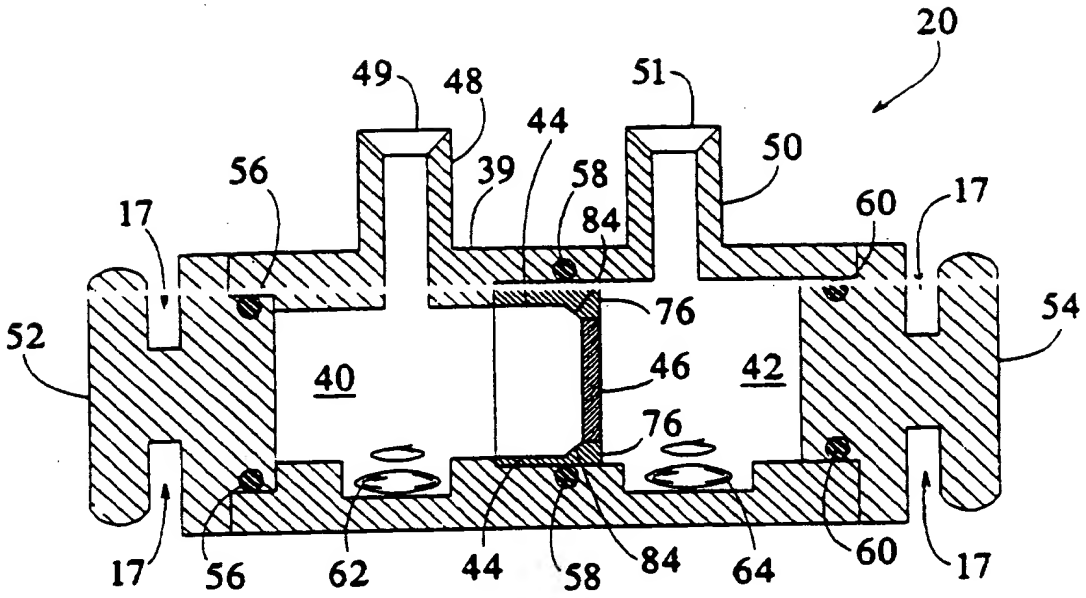
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FIG. 6

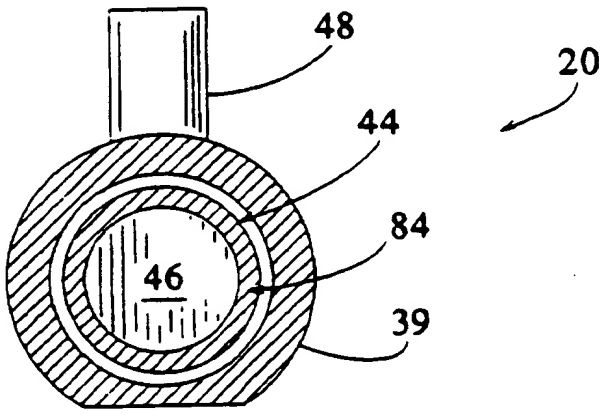
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FIG. 7

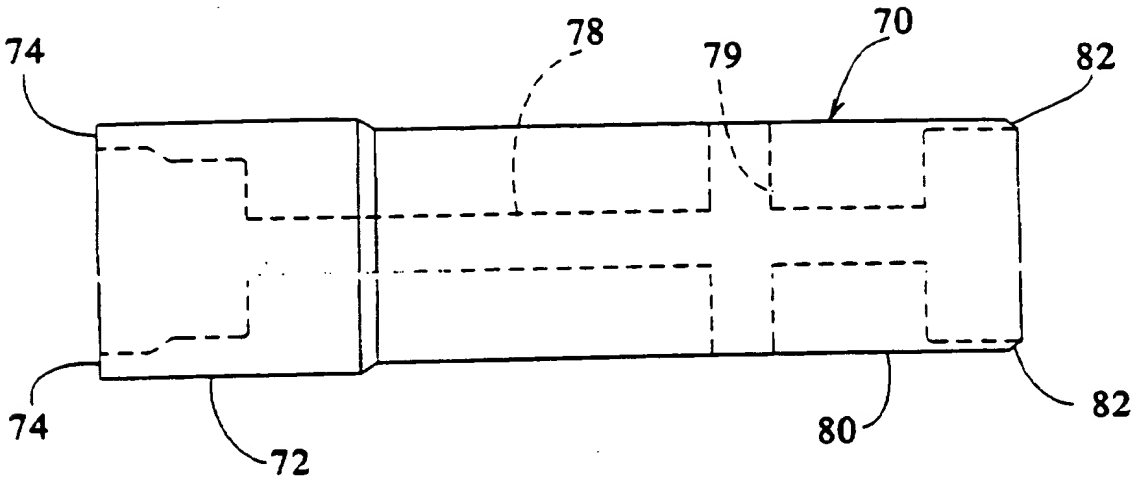


FIG. 8

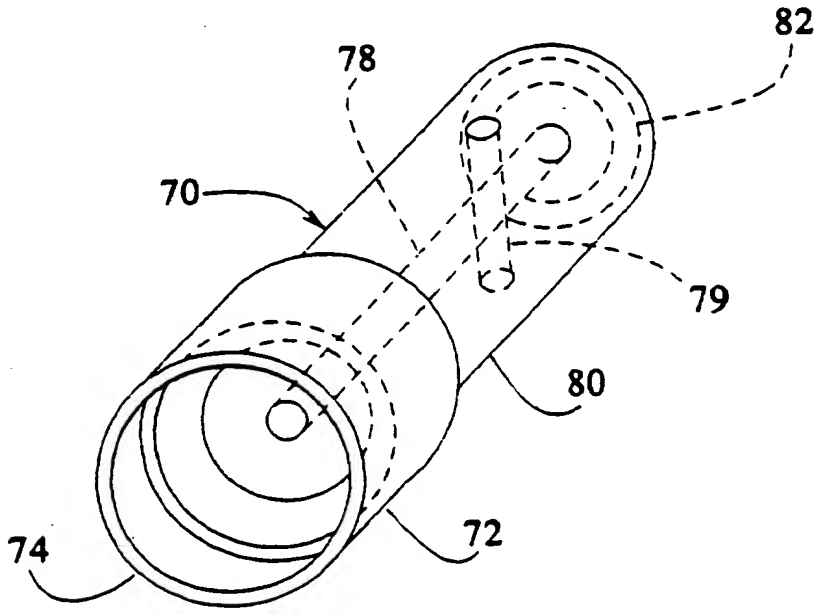
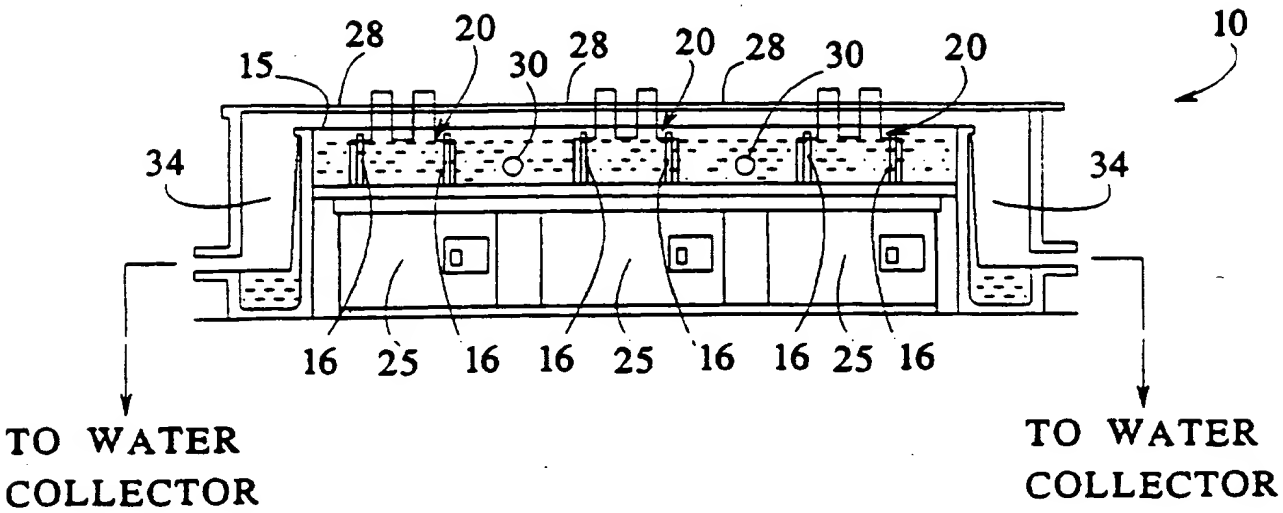


FIG. 9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TO WATER COLLECTOR
TO WATER COLLECTOR

FIG. 10

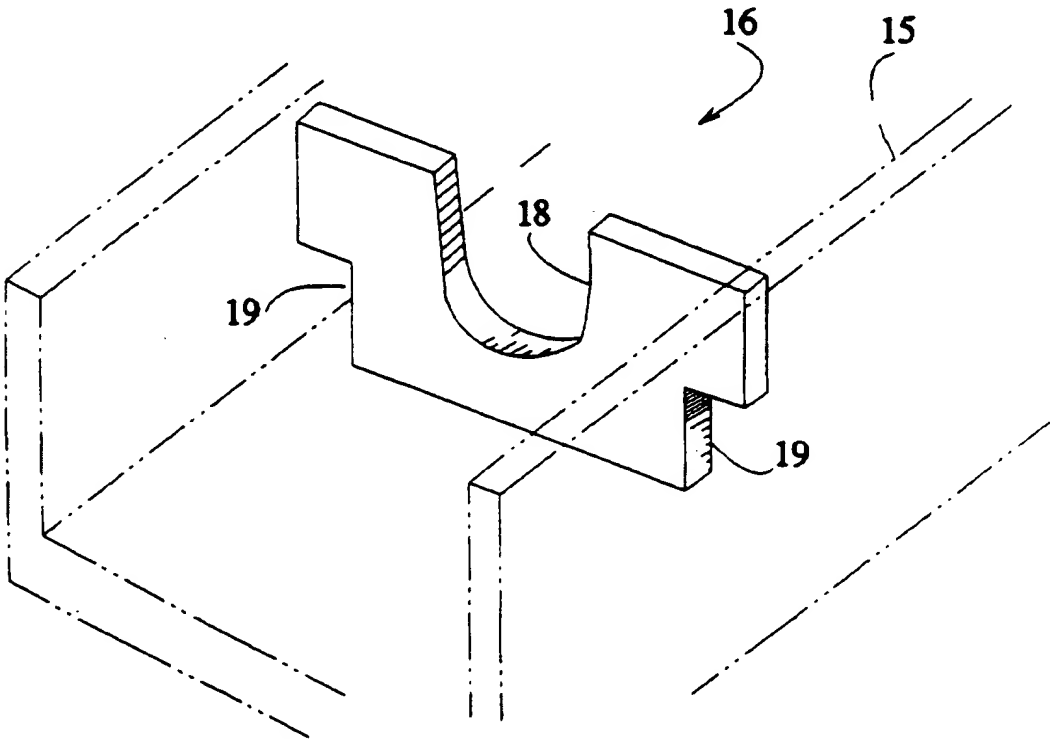
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FIG. 11A

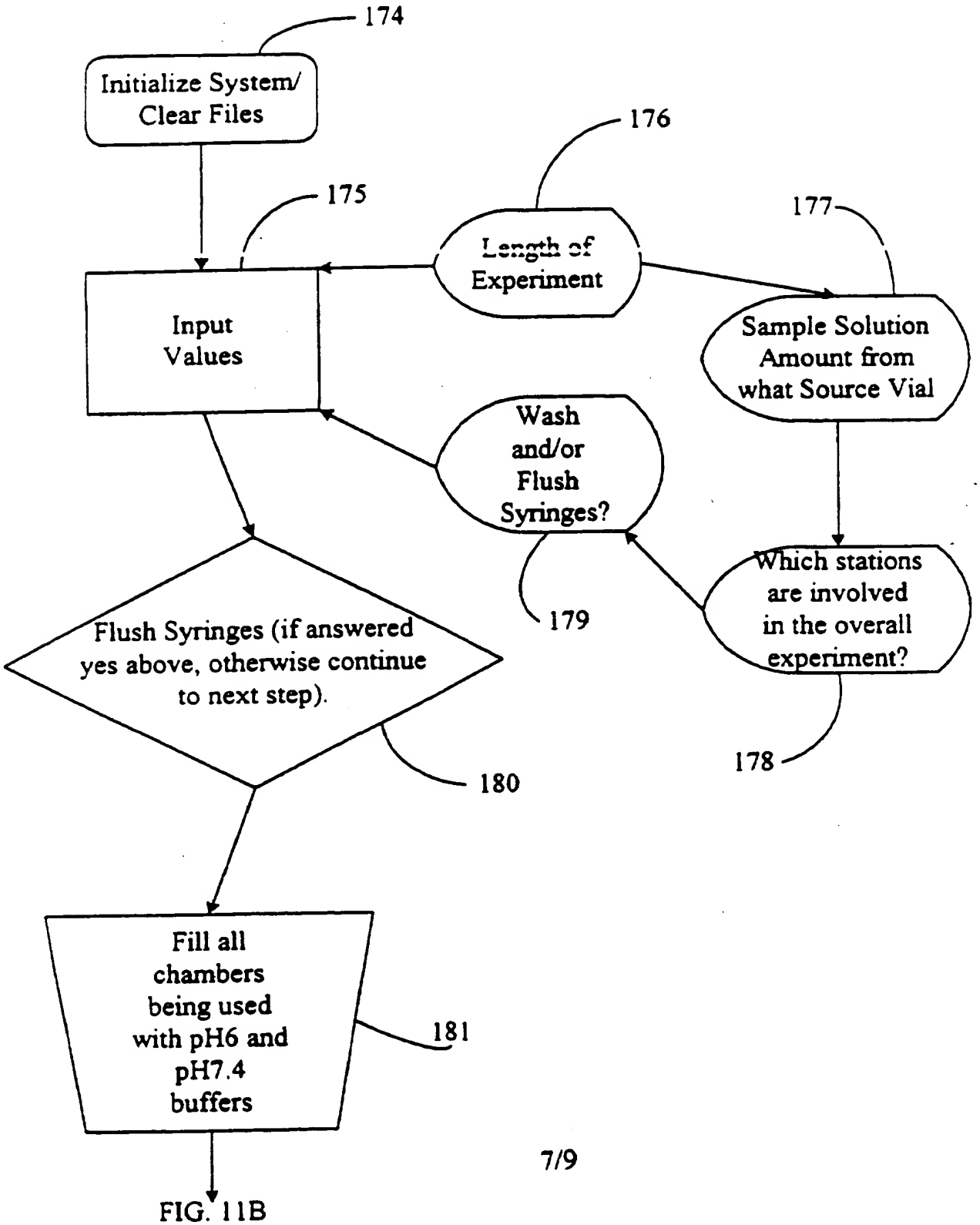
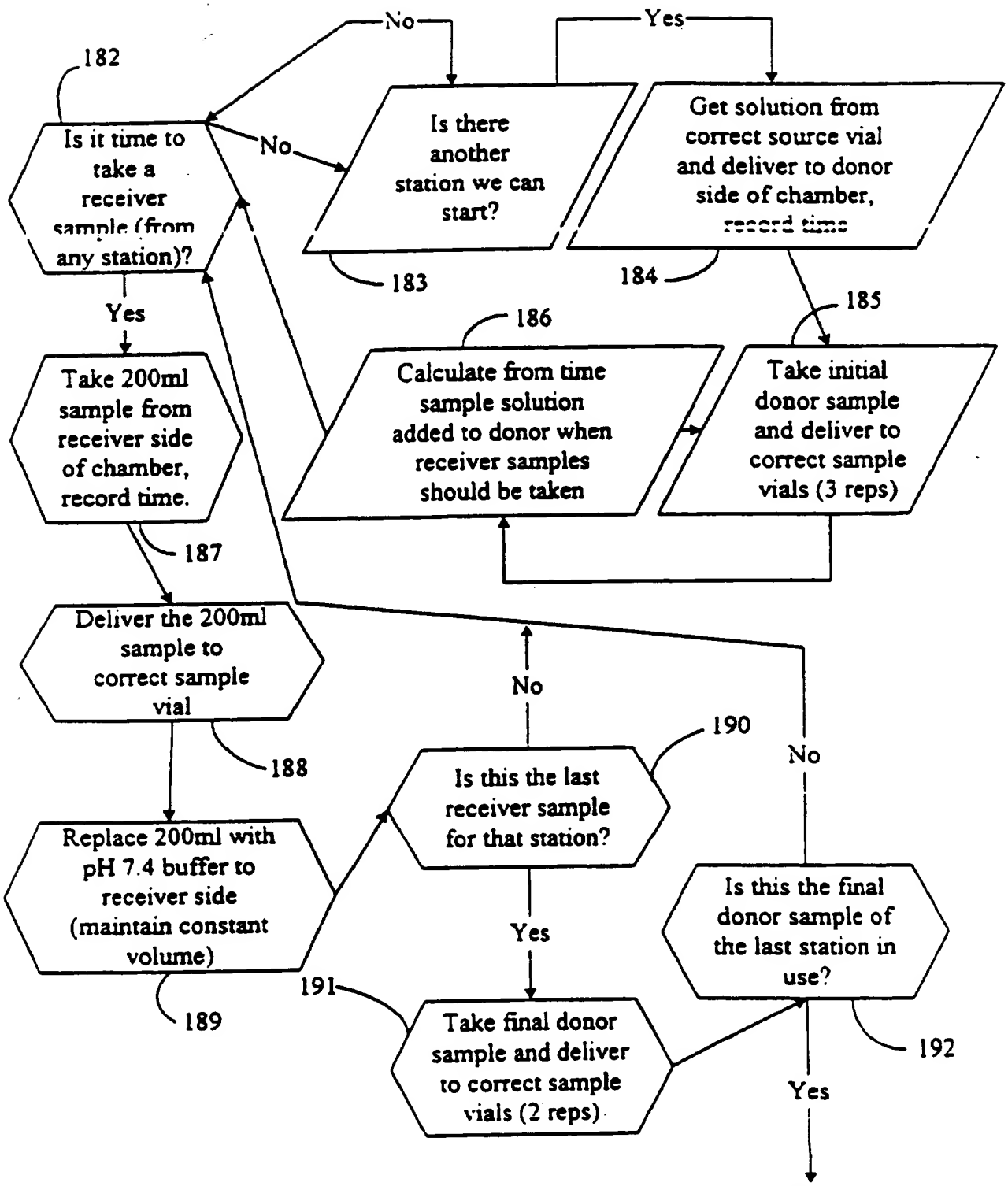


FIG. 11B

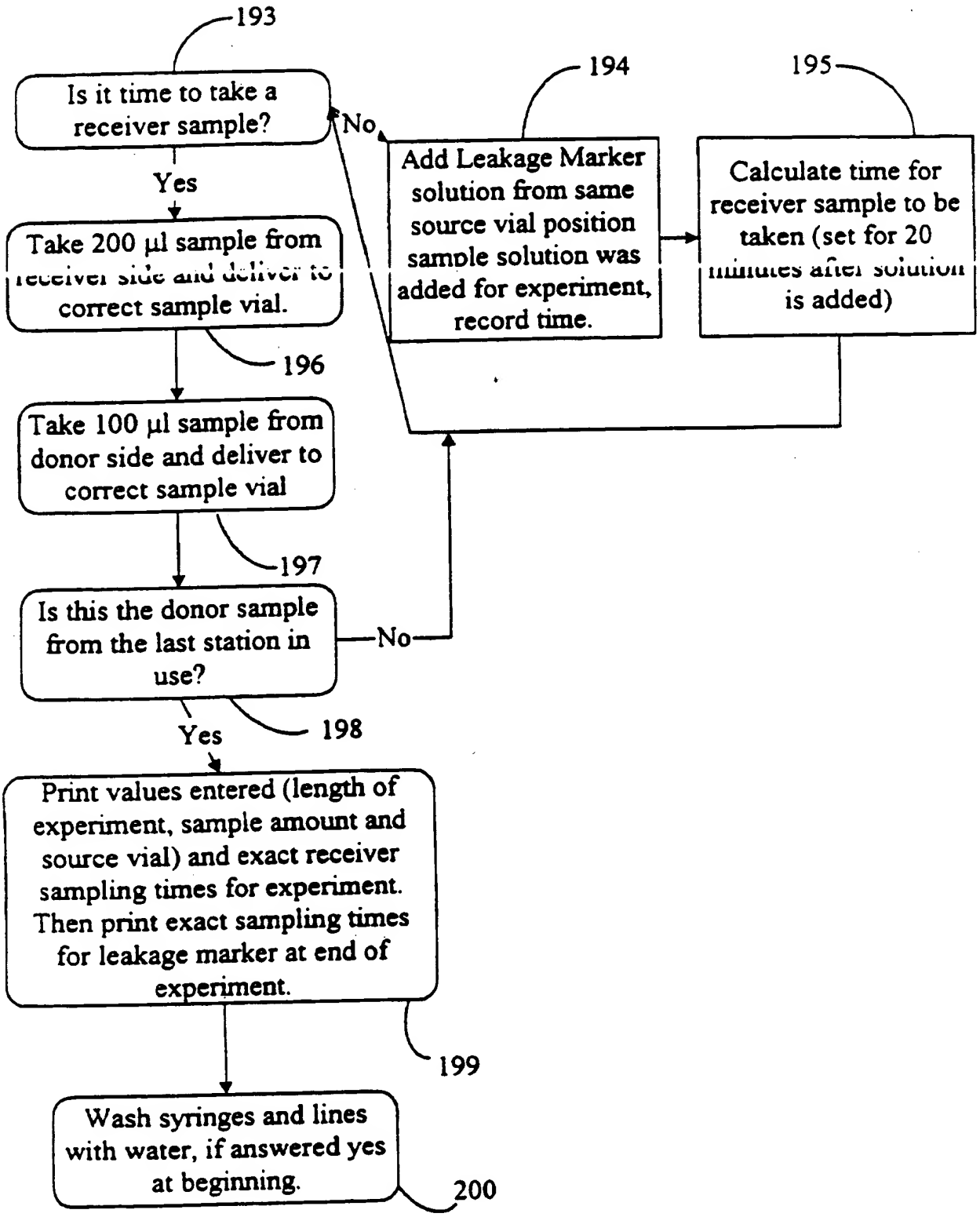
FIG. 11B



8/9

FIG. 11C

FIG. 11C


193
Is it time to take a receiver sample?
No
Yes
194
Add Leakage Marker solution from same source vial position sample solution was added for experiment, record time.
195
Calculate time for receiver sample to be taken (set for 20 minutes after solution is added)
Take 200 µl sample from receiver side and deliver to correct sample vial.
196
Take 100 µl sample from donor side and deliver to correct sample vial
197
Is this the donor sample from the last station in use?
No
Yes
198
Print values entered (length of experiment, sample amount and source vial) and exact receiver sampling times for experiment. Then print exact sampling times for leakage marker at end of experiment.
199
Wash syringes and lines with water, if answered yes at beginning.
200

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 96/17339

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N13/00 G01N33/15

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	WO 83 03901 A (PHARMACONTROL CORP) 10 November 1983 see page 10, line 25 - page 12, line 32 see page 15, line 24 - page 19, line 3 see page 30, line 32 - page 32, line 11 see page 33, line 13 - page 36, line 5; figure 10 ---	1,3-5,8 2,6,7,9, 10,12
A	PHARMACEUTICAL RESEARCH, vol. 8, no. 2, 1991, US, pages 222-227, XP000613852 I.J. HIDALGO ET AL.: "CHARACTERIZATION OF THE UNSTIRRED WATER LAYER IN Caco-2 CELL MONOLAYERS USING A NOVEL DIFFUSION APPARATUS" cited in the application see page 222 - page 223; figure 1 --- -/--	1-4,8,9, 11,14

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 February 1997	21.02.97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Authorized officer Hodson, M

1

Inter nal Applicaton No
PCT/US 96/17339

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 183 760 A (SWEETANA STEPHANIE A ET AL) 2 February 1993 see column 4, line 25 - column 7, line 22; figures	1-4,8,9, 11,14
P,X	US 5 490 415 A (MAK VIVIEN H W ET AL) 13 February 1996 see column 5, line 55 - column 7, line 63; figures	1-6,8,10
A	INTERNATIONAL LABORATORY, vol. 16, no. 9, November 1986, FAIRFIELD CT US, page 56,58,60 XP002024776 J.R.STRIMAITIS: "Pharmaceutical quality control using laboratory robotics" see the whole document	1,6,12, 13
A	INTERNATIONAL JOURNAL OF PHARMACEUTICS, vol. 63, no. 3, 30 September 1990, AMSTERDAM, NL, pages 281-283, XP000613859 P.K. SHAH ET AL.: "A simple diffusion device to study transport across cells cultured on microporous membranes" cited in the application see page 282; figure 1	1-4,10

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 96/17339

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-8303901	10-11-83	US-A-	4578244	25-03-86
		AU-A-	1608983	21-11-83
		CA-A-	1210252	26-08-86
		EP-A-	0106892	02-05-84
US-A-5183760	02-02-93	NONE		
US-A-5490415	13-02-96	NONE		